

R-19-0001 Taxonomic ID

Submitter:

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Subject:

The subject strain is *Parachlorella* STR26155, which is identical to the Recipient strain except for a reporter protein and a nucleic acid signature (see Intergeneric elements).

Recipient:

The wild-type strain is called *Parachlorella* STR00010 which was isolated near the Hawaiian island of Oahu. *Parachlorella* STR00010 was subjected to UV mutagenesis to create the recipient strain STR00012, which has higher biomass productivity than STR00010. This genus has not been seen previously by EPA. *Parachlorella* was shown to be a sister phylogenetic clade closely related to the genus *Chlorella* in phylogenetic analyses using both 18S rRNA and internal transcribed spacer (ITS) data (Krienitz et al., 2004; Kreinitz & Bock, 2012). The submitters used the small subunit rRNA sequence given in Appendix A1 along with the BLAST results to collect closely related *Chlorella* strains (KAS012, SAG211-18, MBIC10088) and confirmed that the *Parachlorella* clade is paraphyletic to the "true *Chlorella*" clade (Lee and Hur, 2012). An independent BLAST analysis confirms the submitter's findings, though additional *Chlorella* matches

Taxonomy

Phylum: Chlorophyta
Class: Trebouxiophyceae
order: Chlorellales
family: Chlorellaceae
genus: *Parachlorella*

Conclusion regarding the recipients:

Based on submitter, EPA, and literature analyses, the identity of the recipient strain as *Parachlorella* strain STR00012 was confirmed and accepted.

Intergeneric elements

There are only two intergeneric changes to the recipient organism claimed by the submitter: a (34bp) loxP site derived from bacteriophage P1 and a nucleotide sequence coding for the green

fluorescent protein (GFP), "TurboGFP™", which is an improved variant of a GFP from a copepod of the order of Calanoida.

The loxP site was used for Cre-Lox Recombination to remove a selectable marker, and remains in the subject genome. This 34bp non-coding intergeneric change will not result in any functional protein.

Copepods are the dominant zooplankton in the world's oceans. The intergeneric addition of TurboGFP will be used for environmental tracking of the subject strain, and has not been reported as toxic (Shagin et al., 2004; <http://evrogen.com/protein-descriptions/TurboGFP-description.pdf>).

References:

- Krienitz, L. and C. Bock. 2012. Present state of the systematics of planktonic coccoid green algae of inland waters. *Hydrobiologia* **698**:295-326.
- Krienitz, L., Hegewald, E.H., Hepperle, D., Huss, V.A.R., Rohr, T. and M. Wolf. 2004. Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (Chlorophyta, Trebouxiophyceae). *Phycologia* **43**(5):529-542.
- Lee, H.J and S.B. Hur. 2012. Comparison between phylogenetic relationships based on 18S rDNA sequences and growth by salinity of *Chlorella*-like species (Chlorophyta). *Fisheries and Aquatic Sciences* **15**(2):125-135.
- Shagin DA, Barsova EV, Yanushevich YG, Fradkov AF, Lukyanov KA, Labas YA, Semenova TN, Ugalde JA, Meyers A, Nunez JM, Widder EA, Lukyanov SA, Matz MV. 2004. *GFP-like proteins as ubiquitous metazoan superfamily: evolution of functional features and structural complexity*, Mol Biol Evol. **21**(5):841-50.

Heim R, Cubitt A, Tsien R. *Improved green fluorescence*, Nature.1995, Feb; 373 (6516): 663–4.